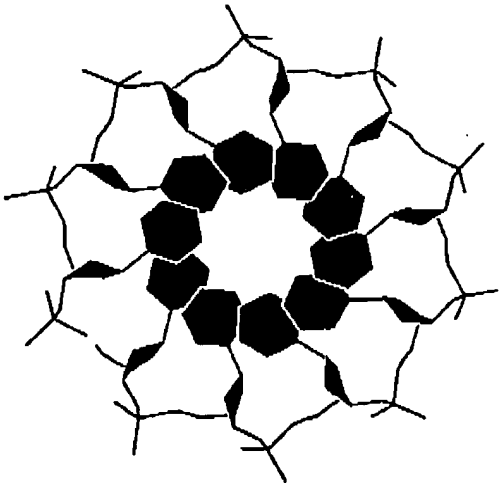
RECEIVED
CENTRAL FAX CENTER
SEP 0 6 2006

Appendix A

(2 pages)

Biochemistry

FOURTH EDITION



Lubert Stryer

STANFORD UNIVERSITY

W. H. Freeman and Company
New York

DNA POLYMERASE I CONTAINS A TEMPLATE-BINDING CLEFT AND A POLYMERASE ACTIVE-SITE CLEFT

This trifunctional enzyme can be split by proteases into a 36-kd *small fragment* with all the original 5′ → 3′ exonuclease activity and a 67-kd *large fragment* (also called the *Klenow fragment*) with all of the polymerase and 3′ → 5′ exonuclease activities (Figure 31-26).

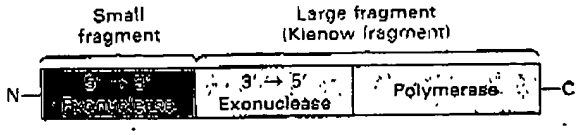


Figure 31-26
DNA polymerase I has three enzymatic activities in a single polypeptide chain.

X-ray crystallographic studies of the large fragment show that it is about 90 Å long. Two prominent clefts that are nearly perpendicular to each other are evident (Figure 31-27A). One cleft serves as the binding site for duplex DNA (Figure 31-27B). The other cleft is thought to contain the catalytic site for polymerization and the binding site for single-stranded template. The 3′ → 5′ exonuclease site is located sufficiently close to the polymerase site to allow the 3′ terminus of the growing chain to shuttle back and forth between them. Thus, polymerization and editing can proceed nearly simultaneously without release of DNA from the enzyme.

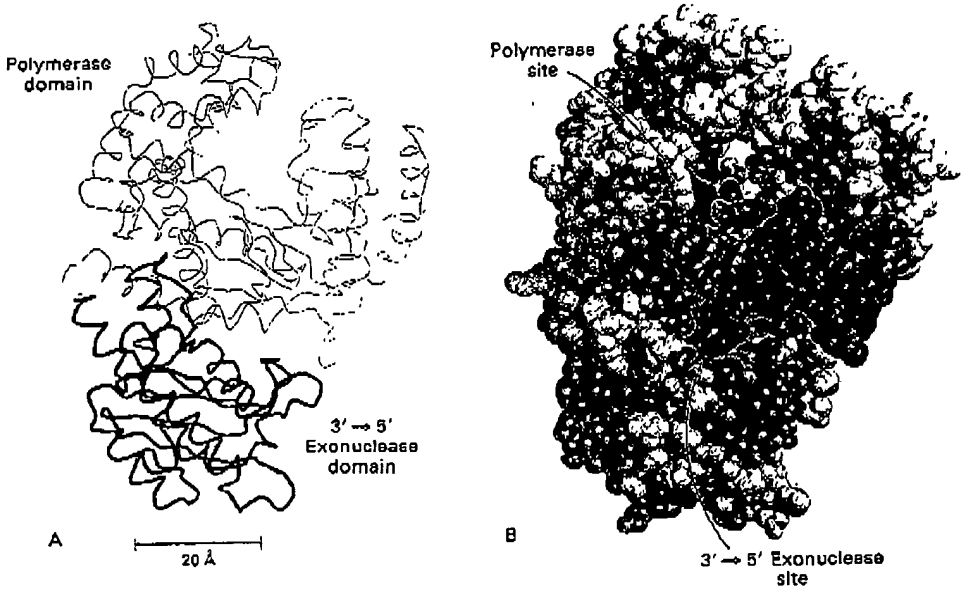


Figure 31-27
Structure of the large fragment (Klenow fragment) of DNA polymerase I. (A) Main-chain trace. The polymerase domain is shown in blue, and the 3′ → 5′ exonuclease domain in yellow. (B) Space-filling model. The primer strand of the bent DNA duplex is shown in red, and the template strand in green. The 3′ end of the primer strand is located in the exonuclease editing site. Several key residues in the polymerase catalytic site are shown in purple. The 3′ end of the primer strand shuttles between these sites, which are about 35 Å apart. [Drawn from coordinates kindly provided by Dr. Thomas Steitz. L.S. Beese, V. Derbyshire, and T.A. Steitz. *Science* 260(1993):352.]